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DIRECT AND INDIRECT ANTIGEN RECOGNITION: THE PATHWAYS TO ALLOGRAFT IMMUNE REJECTION

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1. ABSTRACT

The immune rejection of allografts is mediated by T cells via two distinct pathways: the direct and the indirect pathways. Direct alloresponse to intact donor MHC molecules is ensured by T cells which are polyclonal and directed toward a variety of antigens. This response is highly sensitive to treatment by immunosuppressive drugs including Cyclosporin A. Indirect alloresponse is oligoclonal and involves a few dominant antigen peptides on donor MHC. In contrast to its direct counterpart, indirect allorecognition is thought to be poorly sensitive to blockade by cyclosporin A. It is likely that indirect and direct types of alloresponses play different roles in the physiology of the rejection process. T cell responses occuring via direct allorecognition play a critical role during the early phase of acute graft rejection by sensitizing the host to graft antigens. Alternatively, once such sensitization has taken place, indirect type of alloresponse may become predominant and presumably represent the driving force in the actual destruction of transplanted tissues. In addition, we and others have provided strong circumstantial evidence indicating that secondary T cell responses via indirect allorecognition spread to new determinants on donor MHC and tissue-specific antigens. This phenomenon is likely to play an important role in late and chronic rejection, a major obstacle to long-term graft acceptance in clinical transplantation. Finally, a series of studies have demonstrated that early, pre-transplant treatment with tolerogenic donor-derived MHC peptides can protect the graft from rejection in rodents. Although the mechanisms involved in MHC-peptide-induced tolerance are ill defined, this strategy represents a promising approach for ensuring long-lasting graft acceptance in the absence of widespread immunosuppression. It is now crucial to further explore the mechanims involved in immunogenicity and tolerogenicity of MHC peptides and to initiate clinical studies to evaluate the efficacy of blocking indirect alloresponses in transplanted patients.

2. INTRODUCTION

Transplantation of organs, tissues and cells represents a life saving procedure for a variety of patients affected with incurable diseases. During the past 30 years, progress in surgical techniques and the development of immunosuppressive drugs have rendered possible the allotransplantation (intraspecies grafts) of different organs in patients with minimal risks for early acute rejection. There are still, however, two main barriers to successful large-scale and long-term engraftment in patients: the shortage of available organs and the immune rejection of transplants. After transplantation, a number of donor-derived proteins are recognized as foreign antigens by the recipient's immune system, a phenomenon which results in a potent immunological reaction in which donor cells are rapidly and specifically killed and the graft destroyed (1,2). Despite recent advances in immunosuppressive therapy, such treatment is generally associated with increased risks for infection and neoplasia. In addition, it is often toxic and ineffective in achieving long term graft survival. Chronic allograft rejection is also a critical barrier to succesfull long term survival of allotransplanted tissues and organs in patients. While acute rejection has ben controlled to a large degree with immunosuppressive drugs, there is no current treatment to prevent or block the chronic rejection process. It is due partly to the fact that there is no reliable method to detect early signs of chronic rejection. In most cases chronic rejection is determined using biopsies when the transplanted organs is already severely damaged and its function impaired. Most importantly, the biological mechanisms involved in chronic rejection are unknown. The exact contribution of the immune system to this type of rejection has been a long standing controversial issue in transplantation. However, recent evidence has been provided indicating that early infiltration of the transplants by CD4+ T lymphocytes always preceeds and predicts the events associated with chronic rejection. However, the elements which attract these T cells to the site of the graft are still undefined. Most importantly, the nature of the APCs and the antigen(s) as well as the T cells involved in this process are still elusive, a feature which prevents the elucidation of the immunological mechanisms involved in chronic allograft rejection.

Altogether, this shows that it is crucial to further elucidate the cellular and molecular mechanisms underlying T cell-mediated immune responses to transplantation-

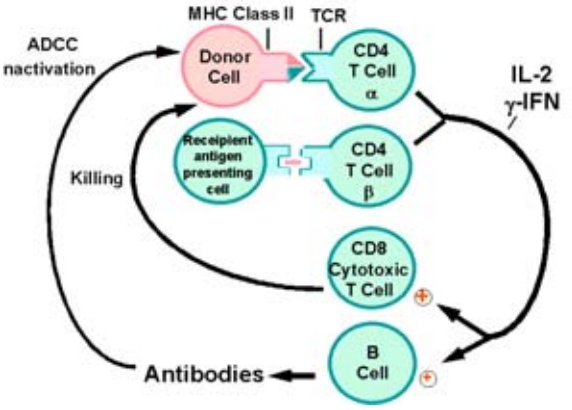

Figure 1: Cellular mechanisms of allorecognition. T cell response to alloantigens on transplanted cells is mediated via two distinct pathways: direct and indirect allorecognition.

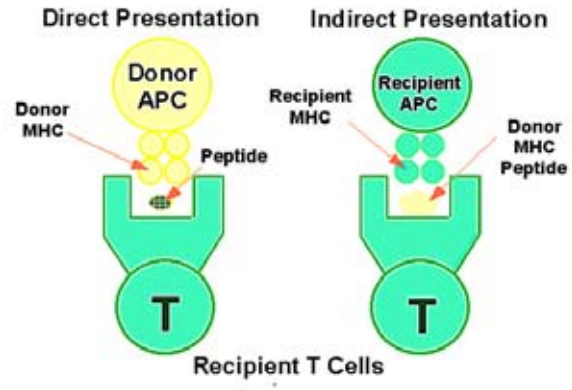

Figure 2: Direct and indirect pathways for presentation of alloantigens in vitro and in vivo. CD4+ T cells recognizing alloantigens in intact or processed forms contribute to the rejection process by providing help to CD8+ cytotoxic T cells and alloantibody-producing B cells.

associated antigens during acute and chronic graft rejection. Based upon this knowledge, we may become able to design antigen-specific strategies to achieve selective and long-lasting immunological tolerance in allotransplantation. In this review article, we examine different aspects of immune responses to transplants and analyze their relative contibution to the rejection of allografts. We also discuss the mechanisms of action of antigen-based strategies designed to accomplish immune tolerance to allotransplants in animal models and discuss their applicability in human clinical transplantation.

3. IMMUNOLGICAL MECHANISMS INVOLVED IN THE IMMUNE REJECTION OF ALLOTRANSPLANTS

3.1. The evidence for two distinct antigen recognition pathways

The immune reaction to donor major histocompatibility complex (MHC) antigens of the graft is known to represent the main hurdle to successful allotransplant engraftment (1,2). The recognition by recipient T lymphocytes of allogeneic MHC molecules on transplanted cells (allorecognition) elicits a potent immunological reaction resulting in rapid elimination of donor cells and the rejection of the graft. Traditionally, allorecognition was thought to occur via only one mechanism: *direct allorecognition* in which T cells recognize determinant peptides on the intact donor MHC molecules displayed on the surface of transplanted cells. However, in the early 1980s, Lechler *et al*. proposed an alternative mechanism in which donor MHC molecules are processed and presented as peptides by self-MHC molecules at the surface of the host's antigen presenting cells (APCs) thereby eliciting a T-cell response which is restricted to the host rather than donor MHC (3). In 1992, we demonstrated that during mouse skin allograft rejection, donor MHC molecules are processed and presented as peptides by the recipient's antigen presenting cells (APCs) *in vivo*, eliciting $CD4^+$ and $CD8^+$ T cell responses which are restricted to the recipient's own MHC molecules (4). This finding has been then extended to both rat and human models and to a variety of organ and tissue transplants and it is now established as a general phenomenon in allotransplantation referred to as *indirect allorecognition* (5-9).

In summary, these studies show that the presentation of peptides either by recipient or donor MHC molecules is an essential element in the establishment of T cell responses to transplantation antigens. Alloantigen recognition occurs via two mechanisms: the direct and the indirect allorecognition pathways. We conclude that direct and indirect T cell-mediated alloresponses are mediated by different APCs and T cells and differ by their cellular mechanisms and therefore represent distinct pathways of allograft rejection (figures 1 and 2).

3.2. Contribution of direct and indirect types of alloresponses to the rejection process

Alloreactive T cells which interact with donor MHC/peptide complexes (direct pathway) are characterized by their high precursor frequency and the diversity of their T cell receptor (TCR) specificities. In contrast, T cells recognizing processed forms of donor antigens associated with recipient MHC molecules (indirect pathway) are directed to a single or a few dominant determinant(s) on donor MHC molecules (11) and they display limited TCR V_b gene usage (11). While direct allorecognition has been known for years to contribute to graft rejection, recently, Auchincloss *et al*. have clearly demonstrated that indirect pathway is also an essential element of the rejection process. In this study, using donor cells from MHC class II knock out mice, it was observed that while direct allorecognition was abrogated, indirect type of alloresponses were preserved and capable of mediating graft rejection (8).

The recent demonstration of the involvement of indirect allorecognition in graft rejection has prompted us to readdress the mechanisms associated with the physiology of graft rejection It is clear that both direct and indirect types of allorecognition participate in T cell responses to donor antigens and contribute to the actual rejection of an allograft. However, the dichotomy between these two pathways has raised a question that is essential to clinical transplant immunologists: What is the relative contribution of each type

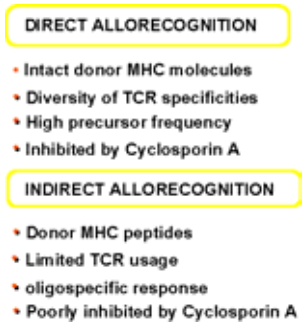

Figure 3: Direct and indirect alloresponses represent two distinct mechanisms of allograft rejection which differ by their clonality, their antigen specificity and their sensitivity to immunosuppressive drugs.

of alloresponses to the actual rejection of an allograft? Initiation of T cell responses to transplanted cells occurs via the recognition of alloantigens presented on donor passenger leukocytes (bone marrow-derived, MHC class II^+: macrophages, dendritic cells) infiltrating recipient's lymphoid organs. Following alloantigen recognition, activated alloreactive T cells infiltrate the graft and secrete lymphokines. For example, interferon g induces MHC class II expression on endothelial and epithelial cells of the graft (12,13). After the passenger leukocytes have left the graft, these endothelial and epithelial cells appear to be the only donor MHC class II-expressing cells in the transplant. Several studies have demonstrated that T cells recognizing donor MHC molecules on these "amateur", defective APCs, become anergized and fail to proliferate and produce IL-2 (14,15). We therefore surmise that recognition of native allo-MHC molecules is only critical for initiation of the alloresponse but it might not be the main mechanism for ensuring the rejection process. Alternatively, the indirect pathway can be triggered by the presentation of donor MHC peptides by recipient-derived "professional" APCs (dendritic cells, macrophages) which infiltrate the graft. The induction of donor class II MHC molecules by gIFN during the secondary inflammatory reactions at the site of the graft should also enhance the processing of donor MHC molecules. Consequently, MHC class II positive inflammatory graft-infiltrating APCs of recipient origin, by processing donor MHC molecules and presenting donor peptides, may provide help for cytotoxic T cell activation and the production of donor-directed antibodies by alloreactive B cells. In conclusion, while indirect alloresponse may not be the driving force in the initial in vivo sensitization of T cells to alloantigens, it may however play a key role in the actual rejection process at the site of the graft.

3.3. Secondary T cell responses in long term and chronic rejection

While some knowledge has been accumulated with regards to the cellular and molecular mechanisms eliciting early acute rejection of allografts, very little is know about later immunological events involved in long-term and chronic rejection process. Both long-term and chronic types of rejection represent major issues in clinical transplantation because of the absence of diagnosis and in most cases the inefficacy of current immunosuppressive agents to block these processes. It has therefore become an important goal in transplantation to design therapies to prevent the onset or block the progression of these forms of allograft rejection. This task necessities the elucidation of the cellular and molecular events involved in these processes. The following describes some recent studies presenting some defined candidate antigens and T cell mechanisms of action which may be involved in the initiation and/or perpetuation of late and chronic rejection.

Based upon the principle that rapidly after transplantation, grafted tissues become devoid of "professional" bone-marrow-derived APCs, we previously hypothesized that long-term and chronic types of rejection were mediated by T cells recognizing antigens on recipient APCs (4). Although this model is still hypothetical, some recent experimental evidence has been provided indicating that indirect allorecognition represents a driving force both in late rejection and in chronic rejection of allografts (16,17). In one study, it has been shown that the chronic rejection process in patients with heart transplants is actually associated with the diversification or antigen-spreading of indirect T cell alloresponses to newly presented determinants on donor MHC proteins. We have recently provided direct evidence that during late rejection, there is diversification or spreading of T cell response to new determinants on donor allogeneic MHC molecules (18,19). Interestingly, we observed that T cell responses can also spread to determinants present on self- i.e. recipient-derived MHC molecules themselves (20). In this case, the alloresponse led to the disruption of immunological tolerance to a self-antigen. Further supporting the potential involvement of this phenomenon in graft rejection, is a study by Fedoseyeva et al. in which we have shown that following heart transplantation, self-tolerance to a cardiac tissue-specific antigen, cardiac myosin, is disrupted (21). We showed that this phenomenon requires initial alloimmune T cell response to donor MHC molecules of the graft. However, once this autoimmune response is engaged it is sufficient on its own to cause the rejection of the graft. Interestingly, cardiac myosin has been previously described as the autoantigen that causes a heart autoimmune disease, autoimmune myocarditis. Our data showed that allotransplantation-induced autoimmunity to cardiac myosin in transplanted mice leads to a histopathology that is indistinguishable to that observed in mice with autoimmune myocarditis. This study suggests that secondary breakdown of self-tolerance to key tissue antigens displayed on the graft may be an essential component of long term rejection of allotransplanted organs.

Altogether, these studies indicate that indirect type of allorecognition by T cells may represent the driving force in chronic and late types of allograft rejection. Such T cell response is presumably directed to newly presented self and donor-derived determinants including formerly silent or cryptic peptides from donor MHC and key tissue-specific antigens shared by both hosts and donors. Further studies will be required to ascertain the role of indirect alloresponses in chronic and late rejection processes. Most importantly, it will be crucial to determine whether secondary alloimmune and autoimmune T cell responses to donor MHC and tissue-specific antigens of the graft are actually responsible for these types of rejection (figure 4).

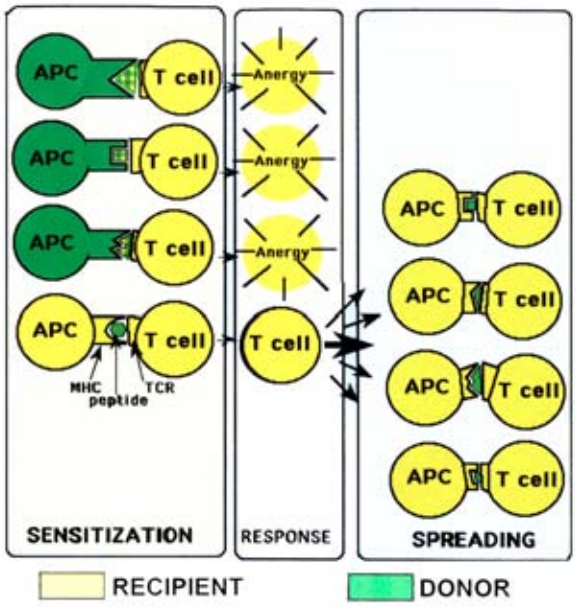

Figure 4: Long term rejection of allotransplanted tissues is associated with spreading of T cell responses to newly presented recipient and donor-derived antigens presented by recipient APCs at the site of the graft.

4. SELECTIVE IMMUNE THERAPY IN TRANSPLANTATION, DREAM OR REALITY?

As we gain insight into the underlying mechanisms that contribute to allorecognition, we may become capable of designing new strategies to manoeuvre the alloresponse toward tolerance or ineffectiveness. The direct alloresponse is characterized by the high frequency of alloreactive T cells recognizing a multitude of antigen peptides and displaying a large variety of T cell receptors on their cell surface (22,23). It is therefore difficult to devise selective antigen- or TCR-based immune therapy to block such a polyclonal T cell response. In contrast, T cell response to donor MHC peptides involved in the indirect allorecognition pathway is mediated by a limited set of T cells responding to a single or a few dominant donor MHC determinants (figure 4). This suggests that selective immune intervention could be designed to interfere with the indirect rejection process, a possibility that has been investigated in our and other laboratories. The following are current working methodologies which use peptides and peptide analogues to achieve T-cell tolerance to alloantigens and long term graft survival.

A number of studies have demonstrated that immunological tolerance to MHC-derived peptides can be achieved by selecting appropriate routes and forms of administration of these peptides in recipient rodents. We have shown that intravenous administration of high doses of MHC class I and II peptides given in saline leads to complete and sustained state of *in vivo* T cell unresponsiveness to these peptides in mice (24,25). Seminal studies by Sayegh *et al*. in 1992 have shown the successful induction of long-term alloantigen-specific allograft survival by intrathymic injection and oral administration of rat recipients with a mixture of peptides corresponding to different polymorphic regions of donor MHC molecules (26,27). In this model, some indirect evidence was provided suggesting that alloreactive T cells may had been inactivated i.e. rendered anergic. Similar observations have also been reported by Oluwole *et al*. in heart transplantation model (28). More recently, we have observed that in a single-MHC mismatched donor-recipient mouse combination, intravenous injection of the dominant donor MHC peptide 12 days prior to skin grafting abrogated the *in vivo* sensitization of alloreactive cytotoxic T cells in recipient mice, and it induced indefinite allograft survival (G. Benichou *et al*., unpublished data). In addition, we showed that intraperitoneal injection of recipient mice with a dominant allo-MHC peptide emulsified in incomplete Freund's adjuvant resulted in a unipolar TH2 type T cell response (29). However, in this model, the effect of immune deviation on graft rejection remains to be investigated. Finally, we recently synthesized a series of analogs of a donor MHC peptide. These peptides display single amino acid substitutions at key T cell receptor contact residues in the sequence of the antigenic peptide. We observed that one peptide analog could induce in vivo and in vitro T cell tolerance to the wild-type MHC antigen peptide (30). Work is in progress to analyze the mechanisms involved in this process and to explore the effect on graft rejection. In conclusion, these studies show that T cell-mediated indirect allorecognition can be manipulated with MHC peptides. Furthermore, different strategies designed to block in vivo anti-donor MHC peptides have proven effective in preventing or blocking allograft rejection in rodents.

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6. REFERENCES

1. Snell, G.D: Studies in histocompatibility. *Science* 213, 172-178 (1981)
2. Dausset, J: The major histocompatibility complex in man. *Science* 213, 1469-1474 (1981)
3. Lechler, R.I., & J.R. Batchelor: Restoration of immnogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. *J Exp Med* 155, 31-41 (1982)
4. Benichou, G., A.P. Takizawa, A.C. Olson, M. McMillan, & E.E. Sercarz: Donor major histocompatibility complex (MHC) peptides are presented by recipient MHC molecules during graft rejection. *J Exp Med* 175, 305-308 (1992)
5. Fangmann, J., R. Dalchau, G.J. Sawyer, C.A. Priestly, & J.W. Fabre: T cell recognition of donor major histocompatibility complex class I peptides during allograft rejection. *Eur J Immunol* 22, 1525-1530 (1992)
6. Liu, Z., N.S. Braunstein, & N. Suciu-Foca: T cell recognition of allopeptides in context of syngeneic MHC. *J Immunol* 148, 35-40 (1992)

7. Shoskes, D.A., & K.J. Wood: Indirect presentation of MHC antigens in transplantation. *Immunol Today* 15, 32-38 (1994)
8. Auchincloss, H.J., R. Lee, S. Shea, J.S. Markowitz, M.J. Grusby, & L.H. Glimcher: The role of "indirect' recognition in initiating rejection of skin grafts from major histocompatibility complex class II-deficient mice. *Proc Natl Acad Sci USA* 90, 3373-3377 (1993)
9. Valujskikh A., D. Matesic, A. Gilliam, D. Anthony, T.M. Haqqi, P.S. Heeger: T cells reactive to a single immunodominant self-restricted allopeptide induce skin graft rejection in mice. *J Clin Invest* 101, 1398-1407 (1998)
10. Benichou, G., E. Fedoseyeva, P.V. Lehmann, C.A. Olson, H.M. Geysen, M. McMillan, & E.E. Sercarz: Limited T cell response to donor MHC peptides during allograft rejection. Implications for selective immune therapy in transplantation. *J Immunol* 153, 938- 945(1994)
11. Liu, Z., Y.K. Sun, & Y.P. Xi: Limited usage of T cell receptor V beta genes by allopeptide-specific T cells. *J Immunol* 150, 3180-3186 (1993)
12. Hayry, P., E. VonWillebrand, E. Partehnais, A. Nemlander, A. Soots, I. Lautenschlager, P. Alfoldy, & R. Renkonen: The inflammatory mechanisms of allograft rejection. *Immunol Rev* 77, 85-142 (1984)
13. Botazzo, G.F., R. Pujol-Borrell, & T. Hanafusa: Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet* 2, 1115-1119 (1983)
14. Lo, D., L.C. Burkly, R.A. Flavell, R.D. Palmiter, & R.L. Brinster: Tolerance in transgenic mice expressing class II major histocompatibility complex on pancreatic acinar cells. *J Exp Med* 170, 87-104 (1989)
15. Gaspari, A.A., M.K. Jenkins, & S.I. Katz: Class II MHC-bearing keratinocytes induce antigen-specific unresponsiveness in hapten-specific TH1 clones. *J Immunol* 141, 2216-2220 (1988)
16. Ciubotariu R., Z. Liu, A.I. Colovai, E. Ho, S. Itescu, S. Ravalli, M.A. Hardy, R. Cortesini, E.A. Rose, N. Suciu-Foca: Persistent allopeptide reactivity and epitope spreading in chronic rejection of organ allografts. *J Clin Invest* 101, 398-405 (1998)
17. Vella J.P., M. Spadafora-Ferreira, B. Murphy, S.I. Alexander, W. Harmon, C.B. Carpenter, M.H. Sayegh: Indirect allorecognition of major histocompatibility complex allopeptides in human renal transplant recipients with chronic graft dysfunction. *Transplantation* 64, 795-800 (1997)
18. Soares, L.R.B., P.L. Orr, M.R. Garovoy, & G. Benichou: Differential activation of T cells by peptide analogues. Influence on auto- and allo-immune in vivo T cell responses. *J Immunol* 160, 4768-4776 (1998)
19. Benichou, G., E. Fedoseyeva, R.C. Tam, K.M. Malloy, & P. Heeger: The presentation of self and allogeneic MHC peptides to T cells: Influence on transplant immunity. *Human Immunol* 59, 540-548 (1998)
20. Fedoseyeva E.V., R.C. Tam, I.A. Popov, P.L. Orr, M.R. Garovoy, & G. Benichou: Induction of T cell responses to a self-antigen following allotransplantation. Transplantation 61, 679-683 (1996)
21. Fedoseyeva, E., F. Zhang, P.L. Orr, D. Levin, H.J. Buncke, & G. Benichou: De novo autoimmunity to cardiac myosin after heart transplantation and its contribution to the rejection process. J Immunol Accepted for publication.
22. Lindahl, K.F., & D.B. Wilson: Histocompatibility antigen-activated cytotoxic T lymphocytes. Estimates of the frequency and specificity of precursors. *J Exp Med* 145, 508-522 (1977)
23. Matzinger, P., & M.J. Bevan: Hypothesis: Why do so many lymphocytes respond to major histocompatibility antigens. *Cell Immunol* 29, 1-7 (1977)
24. Benichou, G., P.A. Takizawa, P.T. Ho, C.C. Killion, C.A. Olson, M. McMillan, & E.E. Sercarz: Immunogenicity and tolerogenicity of self-major histocompatibility complex peptides. *J Exp Med* 172, 1341-1346 (1990)
25. Benichou G., E. Fedoseyeva, C.A. Olson, H.M. Geysen, M. McMillan, & E.E. Sercarz: Disruption of the determinant hierarchy on a self-MHC peptide: concomitant tolerance induction to the dominant determinant and priming to the cryptic self-determinant. *Int Immunol* 6, 131-138 (1994)
26. Sayegh M.H., S.J. Khoury, W.W. Hancock, H.L. Weiner, & C.B. Carpenter: Induction of immunity and oral tolerance with polymorphic class II major histocompatibility complex allopeptides in the rat. *Proc Natl Acad Sci USA* 89, 7762-7766 (1992)
27. Sayegh M.H., N. Perico, O. Imberti, W.W. Hancock, C.B. Carpenter, & G. Remuzzi: Thymic recognition of class II major histocompatibility complex allopeptides induces donor-specific unresponsiveness to renal allografts. Transplantation 56, 461-465 (1993)
28. Oluwole S.F., N.C. Chowdhury, M.X. Jin, & M.A. Hardy: Induction of transplantation tolerance to rat cardiac allografts by intrathymic inoculation of allogeneic soluble peptides. *Transplantation* 56, 1523-1527 (1993)
29. Lehmann, P.V., D. Matesic, G. Benichou, & P.S. Heeger: Induction of T helper 2 immunity to an immunodominant allopeptide. *Transplantation* 64, 292-296 (1997)
30. Soares, L.R.B., P.L. Orr, M.R.- Garovoy, & G. Benichou: Differential activation of T cells by peptide analogues. Influence on auto- and allo-immune in vivo T cell responses. *J Immunol* 160, 4768-4776 (1998)

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